On the Development of the 'Enteronephric' type of Nephridial system found in Indian Earthworms of the genus Pheretima.'

By

Karm Narayan Bahl, D.Sc., D.Phil.

(Merton College, Oxford),

Reader in Zoology, Lucknow University, India.

With Plates 5–7 and 8 Text-figures.

CONTENTS.

1. Introductory.

In a previous paper in this journal (1) I described a new type of nephridial system, which is found in Indian earthworms of the genus Pheretima and which I called 'enteronephric'.

The essential feature of this system is that the numerous septal and pharyngeal nephridia (all micronephridia) are connected with an elaborate system of ducts, which open, not on the surface of the skin but into the lumen of the intestine and other regions of the gut (buccal cavity and pharynx). These nephridia of the 'enteronephric' type co-exist in Pheretima with the integumentary nephridia, which are exceedingly numerous on the inside of the body-wall, and open on the surface of the skin through separate nephridiopores, like ordinary Oligochaete nephridia. Although in my paper I referred very briefly to the possible physiological significance of the discharge of excretory fluid into the gut of this worm, I did not enter, for want of embryological data, upon any discussion concerning the morphological significance of the discovery of the 'entero-nephric' type of nephridial system, in relation to the commonly accepted view, due mainly to Goodrich (**9** and **10**), that all Oligochaete nephridia 'develop centripetally as it were, and quite independently of the coelom and are probably derived from the epiblast'.

While little doubt could be entertained, from a study of the disposition of the nephridial system of the adult worm, with regard to the ectodermal origin of the integumentary nephridia, it was difficult to believe that the septal and pharyngeal nephridia also had a similar origin for two reasons. In the first place, these nephridia have not only no connexion with the body-wall but are connected instead with the inter-segmental septa, which are mesodermal structures; and they open, through an elaborate system of ducts, presumably mesodermal, into the lumen of the gut, the wall of which is partly mesodermal and partly endodermal. In the second place, the septal nephridia differ from the integumentary ones in that the former possess open 'funnels', which are absent in the latter. Although no solenocytes or 'flame-cells' have been found on the integumentary nephridia, the presence of a coelomic funnel in one case and its absence in the other might lead one to ascribe a different origin to the two sets of structures. In fact the connexions of the septal nephridia and their ducts in the adult worm seemed to negative the ectodermal theory of the Oligochaete nephridium, and to point to a mesodermal origin of these nephridia of the new type.

It thus became evident that interesting results would be obtained from a study of the course of development of the nephridial system of this worm, and accordingly I undertook to investigate the problem and the following pages embody the results obtained by me.

The work was carried out in the Department of Comparative Anatomy at Oxford, under the general supervision of Professor E. S. Goodrich, to whom I am very much indebted for the keen interest he has all along taken in my work and for his valuable help and advice.

2. Historical.

The question of the origin of nephridia in Oligochaetes has engaged the attention of many distinguished observers. The early investigators, like Kowalewski (**11**), regarded the nephridium as a tube connecting the coelom with the exterior, and believed that a nephridium arose by a growth of the septal wall of the coelom, that it gave rise to a chain of cells projecting backwards, which eventually fused with the ectoderm and then became hollowed out, so that the whole nephridium is to be looked upon as a 'tail' of the coelom. Moreover, since the first trace of a cavity appears in the region of the funnel and is a prolongation of the body-cavity, the cavity of the nephridium might be said to be part of the coelom. Bergh (**6**) derives the whole nephridium, including the funnel, of Criodrilus and Lumbricus from a single large cell, the 'funnel-cell', lying close to the epiblast, between each successive pair of solid mesoblastic somites. The origin of this 'funnel-cell', from which the whole nephridium develops, has been a matter of considerable dispute. In a later paper on the subject (**7**) Bergh denies the origin of the 'funnel-cell' from the nephric row in Criodrilus and Lumbricus, and asserts that the funnel and the body of the nephridium have a separate and different origin in Rhynchelmis, the upper lip of the funnel arising not from the ‘ funnel-cell ’ (‘ Trichterzelle ’) but from a peritoneal cell.

This view of the mesodermal or the so-called ‘ intraperitoneal ’ origin of nephridia is in strong contrast with that held by Hatschek, Wilson, Meyer, and Vejdovsky (in his later work), which ascribes an ectodermal or a ‘ retroperitoneal ’ origin to the main body of the nephridium and traces the ‘ funnel-cell ’ to the primary nephric row. According to this view the ‘ funnel-cell ’ arises from the primitive cell-row, or nephric cord, formed by the repeated division of one of the teloblasts on either side. In the earlier stages, this teloblast and the nephric cord to which it gives rise lie on the surface of the embryo ; thus the ‘ funnel-cells ’ are epiblastic in origin. From the nephric row one cell enlarges and enters into connexion with each successive segment ; these large cells, arranged metamerically outside and between each pair of somites, are the so-called ‘ funnel-cells ’. In some worms, like Dendrobaena and Lumbricus, the ‘ funnel-cells ’ give off the chain of posterior cells whilst separating from the nephric row, thus remaining for some time in connexion with it. In other cases, such as Criodrilus, the ‘ funnel-cells ’ appear to separate first (**9**).

This view of the superficial origin of nephridia was strongly supported by Goodrich's work (**10**), in which he showed that in certain Polychaetes (e.g. Nephthys) the nephridia do not open into the coelom at all, but terminate internally in a bunch of solenocytes which project into the coelom. He regarded the nephridium as essentially an ectodermic structure comparable with the excretory tube of a Nemertine or of a Platyhelminth. According to him the excretory organs of Oligochaeta are ‘ true ’ nephridia, i.e. tubes originally blind which have acquired secondary communications with the coelom, as distinguished from the ‘ coelomoducts ’, the term he uses for purely mesodermal structures. He points to the co-existence of the genital duct (which is a wide short coelomoduct) and the nephridium in the same somite, in Lumbricus, as evidence that the two structures cannot be homologous with one another (**12**).

The question as to which category (epiblastic or mesoblastic) the Oligochaete nephridia belong has recently been attacked by Staff (**15**), by renewed researches into the mode of their development in Criodrilus.

Staff found 'that in Criodrilus lacuum the mother-cells of the nephridia appear in the ectoderm at the hinder region of the embryo, and here act as teloblasts, giving rise to strings of cells by continuous budding off of smaller cells in front of them, like the mesodermic teloblasts situated internally to them. There are on each side four rows of such ectodermal teloblasts, and the rows of cells to which they give rise become wedged in between the ectoderm and the coelomic mesoderm. The strings of cells destined to give rise to the nephridia are broken into groups, and one group is pushed into each septum which divides one coelomic sac from another. Here each group grows and gives rise to a chain of cells, and this cell-chain becomes hollowed out and forms a tube. Its most internal cell projects into the coelomic cavity between the coelomic cells forming one side of the septum, and forms the greater part of the coelomic funnel of the nephridium. The lower lip of the funnel is constituted by one huge cell belonging to the coelomic wall' (**12**).

According to Staff, therefore, the nephridia develop from the 'retroperitoneal' cell-row, lying lateralwards to 'primitive muscle-fibres' in the manner that this breaks up in segmentally-arranged cell-groups, which project into the body-cavity and are covered over with the peritoneum. The whole nephridium is really ectodermal. The result of Staff's investigation, therefore, is to uphold Goodrich's view.

The earthworm Pheretima (Perichaeta), the development of which I have studied for the purpose of this paper, has all along been held to possess a branched 'plectonephric' nephridial system, a term which has become inapplicable to the system in Pheretima on our further knowledge of it gained recently (**1**). The development of this latter type of nephridia has been investigated by Beddard (**3**) in Octochaetus multiporus, by Vejdovsky (**16**) in Megascolides australis, and by Bourne (**8**) in Mahbenus imperatrix and Perichaeta pellucida.

The earthworm Octochaetus (Acanthodrilus) possesses, in the adult condition in the interior of its body, eight tufts of nephridia in each segment, but a much larger number of external orifices for these nephridia. The funnels are present on these nephridia in the hinder region only and not in the anterior region (**4**). During development, according to Beddard (**3**), the embryo possesses a paired series of organs in each segment, which, as Vejdovsky thinks, are probably the equivalents of the pronephridia of Lumbricus. These paired nephridia of the embryo are, however, provided with well-developed ciliated and functional nephrostomes.

Beddard was not able to follow these paired nephridia to the condition obtaining in the adult, and his work is very incomplete; but he thinks that the nephridia of the embryo are converted into those of the adult, firstly by a temporary cessation of function (?) in a part of the nephridium—the portion nearest the funnel—which is produced by the disappearance of the lumen, and secondly by the active growth of this part of the nephridium, as well as other parts, and by the formation of a fresh series of apertures to the exterior.

Our knowledge of the development of nephridia in the Australian earthworm Megascolides is fairly complete. In the adult condition of this worm the diffuse network of minute excretory tubules is reinforced by the existence of larger paired tubes, one pair to each segment; and these large paired nephridia appear to be in connexion with the smaller tubes. We have, therefore, both the 'meganephric' and the 'plectonephric' systems existing side by side in the same worm. Vejdovsky (**16**) has found that 'in this worm also, during development there is to begin with a pair of nephridia to each segment; these have a funnel, and from the funnel leads a straight duct not perforate; here and there the cells become larger and finally form loops; these loops ultimately increase in size and become complicated coils, the connective point of the original tube degenerating into a mere strand of connective tissue. The last step is the absolute severance of the connexion. Thus it appears, firstly, that the nephridial system of this worm originates from a pair of pronephridia to each segment ; and, secondly, that this becomes broken up into a large number of nephridia, of which one only—the large paired nephridium—retains the funnel ' (**4**).

The development of nephridia in Mahbenus imperatrix described by Bourne (**8**) is remarkably similar to that of the nephridia in Megascolides described by Vejdovsky (**17**). The only difference is that while in the former the funnel is at no stage well developed, is probably never functional, and afterwards entirely degenerates, in the latter the funnel is retained by one pair of nephridia. In fact, the resemblance in the development in the two forms is so great that there is a remarkable similarity between Vejdovsky's diagram (Pl. 32, fig. 5) showing the development of nephridia in Megascolides and Bourne's diagram (Pl. 5, fig. 39) showing the same in Mahbenus.

From the foregoing account of the history of our knowledge of the development of nephridia in earthworms we arrive at three more or less definite broad conclusions. The first is with regard to the fundamental problem of the ultimate origin (ecto- or mesodermal) of the Oligochaete nephridium. As we have seen, there is an overwhelming amount of evidence to show that the nephridia in Oligochaetes are certainly ectodermal.

Secondly, in all forms with the so-called ' plectonephric ' system, studied so far, this adult condition is preceded in the embryo by a condition of paired pronephridia in each segment. In the third place, the adult condition of diffuse micronephridia is derived by the breaking up into separate loops of the embryonic pair of pronephridia, the original funnel either being retained by one of the nephridia in each segment or degenerating altogether.

The present work on the development of nephridia was undertaken to find an answer to the following questions :

1. Are all the three types of nephridia in Pheretima ectodermal in origin ?

2. If they are ectodermal, how do the septal nephridia with their ducts come to lose all connexion with the body-wall and be associated with the septa and the gut, which are mesodermal and endodermal structures respectively ?

3. Is the adult condition of nephridia preceded by a 'meganephric' or paired condition in the embryo ?

4. If so, how is the adult condition derived from the embryonic condition ?

5. Do facts of development throw any light on the phylogeny of the Oligochaete nephridial system ?

3. The Cocoon.

The egg-capsules or cocoons of Pheretima do not differ in any essential particular of structure from those of Lumbricus, Allolobophora, or Acanthodrilus, previously described by Vejdovsky (**16**) and Beddard (**3**) ; but I am recording here my observations on the cocoons of this worm to bring out their special characters.

I have no observations to offer on the mode of formation of this structure in Pheretima, but I have no reason to doubt that it is formed in much the same way as in all the other genera where cocoon-formation has been carefully studied, and that the clitellum alone is concerned in its production.

Although the cocoons vary somewhat in size, they are very much smaller than those of Lumbricus. On an average they are about 1·5 to 2 mm. by 1·8 to 2·4 mm., i.e. about one-third the size of the cocoons of Lumbricus.

The cocoons are light yellow or olivaceous in colour, the empty cases having a clear transparent olive colour. In form they are more or less rounded in shape and give a distinctly swollen appearance, the two ends being drawn out into very short fibrous appendages.

My observations on the time of egg-laying are based on two species of Pheretima, namely P. posthuma and P. rodricensis. The cocoons of the first species were found by me at Allahabad (India) in spring and summer months

(March to June) out of doors in moist places in the surface layers of the soil in abundance, but during the rains (July and August) they were very rare. My friend Mr. B. K. Das has since informed me from Allahabad that he has been able to collect cocoons of earthworms (not necessarily of Pheretima) in the months of November, December, January, and February ; and he rightly suspects that egg-laying continues almost throughout the year. Of course the number of cocoons found in the winter months is very small, since the surface layers of the soil get very dry on account of the prolonged drought, and the worms go deep into the soil and are themselves difficult to obtain.

As regards the cocoons of P. rodricensis,[1] my observations are based on worms kept in captivity in garden-pots in a hot-house. In order to make sure of the specific identity of my cocoons I kept worms of this species in sterilized earth, to which decaying leaves previously sterilized were added from time to time. From a number of garden-pots containing these worms I could obtain cocoons in any number containing embryos at various stages of development throughout the year. The statement is usually made in text-books that ' egg-capsules are formed in spring or early summer and the young worms grow mainly during the summer months. Sometimes large clusters matted together may be found in autumn packed away under clods or in banks where there is a favourable condition of moisture '.[2] Wilson (**18**) says, ' egg-laying seems in special cases to continue throughout the year, though it is most active in the spring and summer months. I have found the capsules of Lumbricus foetidus out of doors in nearly every month of the year, but in mid-winter they are only found in decomposing compost-heaps where the temperature is maintained at a tolerably high point '. From these authorities and from my own observations I am inclined to believe that the time of egg-laying depends

[1] I am indebted to Col. J. Stephenson of the University of Edinburgh for identification of this species.

[2] Osborn, ' Economic Zoology ', New York, 1908, pp. 110-11.

very largely on the external conditions—temperature, moisture, and the richness of soil. My garden-pots containing the worm were kept quite damp ; the temperature of the hot-house was always about 60° F. and the soil was frequently ' manured ', so to speak ; and it is no wonder, therefore, that under these artificial conditions cocoons were obtained at all times of the year. In nature these conditions are best fulfilled in spring and early summer, and hence we get the greatest activity in egg-laying in these months, although it seems that it does not stop altogether at other times of the year.

I have opened hundreds of cocoons of Pheretima and feel justified in considering, as a rule, there is only one embryo in a cocoon. Occasionally one comes across two embryos in a cocoon of a very young age, and only once did I see three embryos in one cocoon. In fig. 24, I have tried to represent three typical stages of the embryo of Pheretima in their natural size (the external segmentation of the body, although complete throughout, cannot be made out with the naked eye and is therefore not represented).

The rate of development is very much slower in Pheretima than in Lumbricus. Wilson (**18**) found that in laboratory cultures the young worms (Lumbricus) made their escape from the capsule in about two or three weeks. Beddard (**3**) judges that the shortest time in Acanthodrilus can hardly be less than five or six weeks. In Pheretima the rate is even slower than that in Acanthodrilus, and I cannot put the shortest period at less than eight weeks in this case.

Beddard (**3**) found that the albuminous fluid filling the cocoon in Acanthodrilus, as in Lumbricus rubellus, was milky and opaque while the shell was transparent ; in Pheretima, however, the albuminous substance of the cocoon is perfectly clear and transparent like its shell, so that under a binocular microscope I could always see, by transmitted light, the embryo inside the cocoon without opening it, and it was thus very convenient to be able to know roughly the size and age of the embryo before opening it.

Vejdovsky and Beddard speak of two perfectly distinct membranes forming the shell of the cocoon. I have not been able to see these two membranes in the case of Pheretima cocoons, the shell of which seems to me to be single-layered.

4. General Outline of the Development of Nephridia in Pheretima.

The three sets of nephridia of Pheretima, namely, the integumentary, the septal, and the pharyngeal arise in the embryo at successive stages of its development. In order to elucidate, therefore, the development of the whole nephridial system consisting of these three distinct series of nephridia and their ducts, it is necessary to examine a large number of embryos of widely different ages. The work is rendered laborious and difficult on account of three facts: firstly, that each type of nephridium develops independently of the other—these several types are not derived one from the other; secondly, that the nephridia of the three series develop at different ages and in different positions in the embryo; and thirdly, that each series consists of numerous nephridia that go on developing for a long time even after the embryo has left the cocoon. But before going into the details of each stage of nephridial development, I shall provide here an outline sketch of the development of the elaborate excretory system of this worm.

Leaving aside the transitory excretory cells the earliest beginnings of permanent nephridia appear in this worm, as in Lumbricus (**18**), Rhynchelmis (**16**), and Criodrilus (**15**), as teloblasts lying on the surface of the embryo, ventral to the mesoblastic bands and in front of the mesodermal pole-cells. While these teloblasts form part of the surface epiblast in very young embryos (300 μ long), they soon sink below the surface and come to lie between the definitive ectoderm and the mesoderm. Strings of cells are budded off from and in front of these ectodermal teloblasts, and it is these cell-rows (nephroblasts) that form the material foundation ('Anlage') from which are derived all the future nephridia.

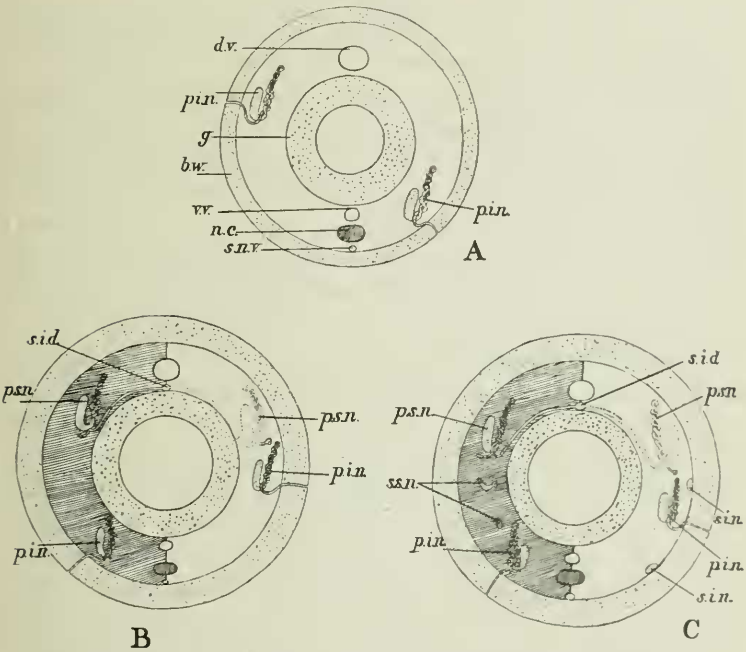
These strings of nephridial cells aggregate later into groups that lie opposite and a little posterior to the places where the intersegmental septa join the body-wall. These groups of cells, situated underneath the mesodermal peritoneal membrane (' somatopleure '), proliferate to form masses of cells, which, as they grow, begin to project into the coelomic cavity. These constitute the nephridial rudiments. They carry with them in their growth the sheet of peritoneal membrane, which now forms an enveloping sheath over these unformed nephridia (fig. 9). In longitudinal sections of an embryo, about 4 mm. in length, these embryonic nephridia are seen for the most part as solid clup-shaped masses, lying in the anterior part of each coelomic chamber, a pair in each segment of the body except the first two. While the first two segments are devoid of nephridia and the greater part of the embryo possesses solid nephridial masses, some of the anterior segments (seventh and eighth, for example) have fully-formed nephridia with the characteristic shape and the intra-cellular canals of the adult organ.

In preparations of whole embryos of suitable age, flattened after opening them through the mid-dorsal line, we can see the rudiments of these primary nephridia as elongated masses lying posterior to the septa towards the hind end of the embryo ; but, as we examine the segments in front, we get the nephridia in all stages of development in the same embryo, since development proceeds antero-posteriorly. We may note here that these nephridia have no connexion with the septal partitions, and consequently a ' septal funnel ' is never formed at any stage of development of this primary pair of integumentary nephridia.

At this stage of development (4 mm. long) the embryo exhibits a typical meganephric or paired condition like that of the adult Lumbricus, having a pair of ' true ' ectodermal nephridia in each segment (Text-fig. 1 A). This marks the first stage in the development of nephridia in Pheretima, which comprises the developmental history from the first appearance of teloblasts up to the formation of a pair of primary integumentary nephridia in each segment.

In the second stage that follows we have the appearance and development of the primary pair of septal nephridia in each

TEXT-FIG. 1.



Diagrammatic representation of the three stages of development of the nephridial system in *Pheretima*. *A* represents a diagrammatic section of an embryo about 4 mm. in length, showing the paired condition of nephridia (meganephric stage). *B* represents a stage at which the embryo has two pairs of nephridia, in each segment, a primary integumentary pair of the first stage and a primary septal pair. *C* shows the formation of secondary septal and integumentary nephridia. In *B* and *C* the intersegmental septum is shown on the left half. *b.w.*, body-wall; *g.*, gut; *d.v.*, dorsal blood-vessel; *v.v.*, ventral blood-vessel; *s.n.v.*, subneural vessel; *n.c.*, nerve-cord; *p.i.n.*, primary integumental nephridia; *p.s.n.*, primary septal nephridia; *s.i.n.*, secondary integumentary nephridia; *s.s.n.*, secondary septal nephridia.

segment of the body behind the first fourteen. As these nephridia begin to appear before all the integumentary ones of the first stage have attained to their full development and size, we have the later development of integumentary nephridia going on side by side with the appearance and growth of septal nephridia, so that we have an overlapping, so to speak, of the first and second stages. The rudiments of septal nephridia appear in two rows, one on each side of the dorsal vessel. The latter in embryos is single anteriorly but double for the greater part of the posterior portion, and the earliest rudiments of the septal nephridia recognizable in whole preparations lie on both sides of this double dorsal vessel (Text-fig. 4). But while the integumentary nephridia vary in their topographical position from segment to segment, lying close to and away from the nerve-cord alternately, the septal ones lie in two straight rows, nearer the mid-dorsal than the mid-ventral line.

As their name implies, the septal nephridia develop on the intersegmental septa and, in sections, can be seen to lie just internal to the commissural that connects the dorsal with the subneural blood-vessel. As a septal nephridium develops, the pre-septal portion elongates to form a long narrow tube ending in the funnel, the body of the nephridium, lying in the coelomic cavity behind the septum, develops the limbs and loops of the adult organ, while the terminal duct elongates to run along the septum, parallel and internal to the commissural vessel, to meet its fellow into the supra-intestinal duct mid-dorsally.

These pairs of nephridia of the second stage differ from the primary integumentary nephridia in that the former develop on the septal wall and have no connexion with the body-wall from the very beginning, and that they develop a septal funnel. Thus we see that septal nephridia are not derived from integumentary ones, and have no connexion with them except that, as will be shown later, both types can be traced to the same source.

When the embryo has developed a pair of septal nephridia in each segment we get to the end of the second stage. At this stage the embryo possesses, in each of its typical segments, two pairs of nephridia, an integumentary pair and a septal one, the former opening to the exterior on the body-wall and lying alternately dorsally and ventrally, and the latter opening into the supra-intestinal duct and lying dorsally throughout (Text-fig. 1 B). The vertical ducts leading from the supra-intestinals to the lumen of the gut at each intersegmentum are also formed at the end of this stage.

In the third stage we have the development of secondary nephridia, integumentary and septal. These begin to appear at a rather late period of development of the embryo, when it is almost fully formed and is about to come out of the cocoon. The circlets of setae are completely formed in all the segments of this age, and while rudiments only can be seen of septal and integumentary nephridia towards the posterior end, we find them in various stages of development anteriorly.

These secondary nephridia of both types appear independently of the primary pairs of their segments. The integumentary ones appear earlier than the septal, since towards the posterior end we find segments with rudiments of secondary integumentary nephridia but with no traces of secondary septal ones. In both cases these nephridia in their initial stages are lumps of cells having no connexion with the primary nephridia.

The septal secondary nephridia appear immediately ventral to the primary pair, and develop very much in the same way as the primary pair, their terminal ducts running dorsalwards on the septa and meeting the ducts of the primary nephridia. Some of these nephridia develop a pre-septal funnel like the primary nephridia, but others, as shown in fig. 15 A, develop the funnel in the same segment in which they lie. In this way we get two kinds of nephridia, one kind with pre-septal funnels and the other with funnels in the same segments as the nephridia. Subsequent pairs of nephridia develop similarly, and we get the formation of the septal canals by the union of the terminal ducts of secondary nephridia with those of the primary ones.

The secondary integumentary nephridia first appear on the body-wall as masses of cells lying beneath the somatic layer of the coelomic epithelium. They do not arise strictly in pairs like the primary nephridia, but have a more or less scattered arrangement. They may appear either on the dorsal or on the ventral side of the primary nephridia (Text-fig. 1 c). They arise in connexion with the setal sacs, and one can very often see a string of cells running from the rudiment of a secondary nephridium in the coelom to the epidermis alongside a setal sac.

Text-fig. 2.

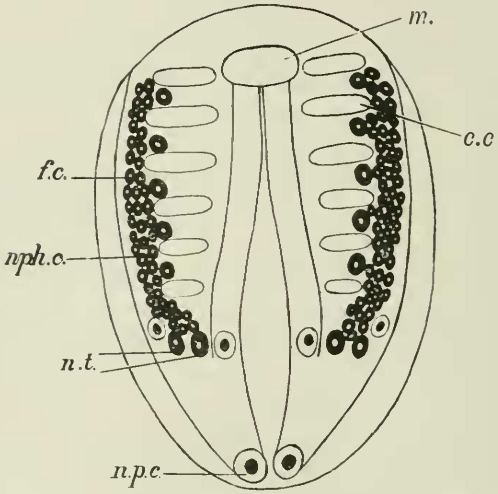


Diagram showing the formation of nephridial rows of teloblasts and the intersegmental position of large 'funnel-cells'. *m.*, mouth. For lettering see Text-fig. 3.

The pharyngeal nephridia of the fourth, fifth, and the sixth segments make their first appearance in a manner similar to the primary integumentary nephridia, although the former lag behind in development, the integumentary nephridia growing faster than the pharyngeal. Amongst the pharyngeal nephridia themselves the nephridia of the sixth segment develop faster than those of the fifth, and these, in their turn, faster than those of the fourth. The first pair in each of these segments originates from the ectodermal nephridial row, and has a long terminal duct which meets the wall of the pharynx ventrolaterally. Secondary pharyngeal nephridia are formed distal to the primary ones as buds on the ducts of the primary pairs of nephridia (fig. 19 and Text-fig. 3).

5. Development of the Primary Integumentary Nephridia.

Although I observed short intracellular canals in certain ectodermal cells of very young embryos (gastrulae) in the living condition, and believed these cells to be of the nature of larval excretory cells, I could not definitely locate them in preserved and stained embryos, nor could I examine and arrive at any definite result about these cells in sections. I shall, therefore, confine myself to the development of permanent nephridia alone.

As already indicated, the first set of nephridia to make their appearance in Pheretima are the ectodermal primary nephridia, a pair in each segment. At a stage of development when the embryo has fifty to fifty-five clearly-defined segments and is about 4 mm. long, we can easily see some of the anterior segments (seventh and eighth, for example) possessing a pair of fully-formed nephridia opening on the surface of the body-wall. Each of these segments, at this stage, resembles in this respect a segment of the adult Lumbricus, and we may even call this stage of development of the nephridial system of Pheretima the 'meganephric' stage.

The early history of these nephridia is very similar to that described in Lumbricus, Criodrilus, and other worms by previous writers. In an advanced gastrula in which the mesodermal bands are well formed and in which cavities are beginning to appear, we can recognize the earliest beginnings of nephridia. On examining such an embryo, when it is still a rounded sphere and has not begun to elongate, as for example the one shown in fig. 1, which is about 140 μ in diameter, we see the mesoblastic bands diverging from the two large mesodermal pole-cells lying at the future posterior end. These bands lie along the two sides of the ventral surface of the embryo, and, on careful focusing, we can also see the beginnings of five or six coelomic cavities in each of the two bands. On examining, however, the surface epiblast covering these mesoblastic bands ventrally, we can distinguish four rather large and rounded cells on each side, called the teloblasts. These teloblasts lie a little way in front of the pole-cells: the three ventral ones lie six or seven cells in front of the pole-cells as seen in longitudinal sections (fig. 2), while the fourth, the lateral teloblast, lies a little farther forward than the rest. In these very young embryos (figs. 1–4) the teloblasts form part of the surface epiblast, but can be easily distinguished from the adjacent epiblastic cells both by their larger size and by the fact that their nuclei are free from granules surrounding the nucleolus and thus give an appearance of greater transparency as compared with the nuclei of the other cells. Of these four teloblasts on each side the one near the mid-ventral line is the neuroblast, going to form the nerve-cord of the adult, the two lying outside the neuroblast are the nephroblasts, which go to form the nephridia, while the outermost and dorsal is the lateral teloblast which lies just outside and dorsal to the coelomic sac on each side at this stage of development of the embryo (fig. 4). In a series of transverse sections of an embryo, about 300 μ in length, from which figs. 3, 4, and 6 are taken, we can follow these four teloblasts forwards as they bud off rows of cells in front. The rows of cells in front of the teloblasts can be followed for a long way in young embryos. Concerning the nephridial cells in continuation with the nephroblasts, we have to note that while the nephroblasts are large cells and occupy the whole thickness of the epiblast, the nephridial cells in front are small and come to lie deep in the ectoderm. They can be seen distinctly marked off by a sort of boundary line from the definite epiblast, which is very thin at places where these nephridial cells occur. These cells are thus embedded in the ectoderm, as shown in figs. 3, 4, and 6, but they have not yet formed a separate layer of their own.

The next step in the development of the nephridia, which is slower than that of the nerve-cord, is that the nephridial teloblasts and the rows of cells in front of them sink beneath the ectoderm and come to form a separate and distinct layer of their own, between the ectoderm on the outside and the mesodermal lining of the coelomic cavities on the inside. In longitudinal sections of young embryos (fig. 5) this layer towards the posterior end gives the appearance of a kind of string of nephridial cells. The large nephridial teloblast together with a row of smaller cells lying in front of it form the definitive nephridial layer. The transition from the previous stage can be well appreciated by comparing the position of the nephroblasts and the nephridial cells in figs. 2, 3, and 4, where they are superficial, with the deeper position occupied by them in figs. 5 and 7. This nephric cord is single-layered in the beginning and remains so for a long time at the posterior end, but its cells soon begin to multiply and proliferate opposite and behind the intersegmental septa which divide one coelomic sac from another, so that we get groups of these nephridial cells situated at intersegmental intervals. The cells of these intersegmental nephridial groups multiply here beneath the peritoneal lining of the coelom. and the cells tend to travel backwards towards the middle of the segment. Some of these nephridial cells push their way into the septa between the two apposing walls of the adjoining coelomic chambers (figs. 7 and 13).

We thus get these intersegmental nephridial masses segregating into two separate groups, one keeping its ' retroperitoneal ' position while shifting backwards and multiplying rapidly, the other consisting of very few cells which make their way into the septa and lie between the two sheets of peritoneum forming the two faces of the septa. In earlier stages—or what amounts to the same thing, in the posterior segments of the embryos—we can see, in longitudinal sections, one nephridial group in each segment lying at the posterior of the two angles formed by the septum with the ventral body-wall (fig. 8). In later stages, or in the more advanced anterior segments, the segregation into two groups becomes quite evident. The group consisting of a few cells caught in between the two septal sheets, leads, to anticipate matters, to the development of septal nephridia, which we shall speak of in the next part of the paper, while the other group consisting of a number of cells lying beneath the peritoneum and immediately posterior to each intersegmental septum, is the rudiment of the primary pair of integumentary nephridia. We shall now consider the details of development of these integumentary nephridia.

This group of cells beneath the peritoneum is the 'retro-peritoneal' group of Meyer (**13**) and forms the forecast of the whole primary nephridium of the first stage. The cells of this group separate away from the septum, divide and proliferate so as to bulge out as solid masses into the coelomic cavities, as shown in fig. 8, *c*. They carry with them their peritoneal covering which forms a thin sheath round these solid nephridia. The growth is not only vertical but also horizontal, and the nephridial rudiment besides increasing in thickness and projecting into the coelom also extends laterally, so that in a preparation showing the body-wall of an embryo flattened we get a pair of deeply-staining elongated solid masses of cells lying immediately behind each septum as shown in fig. **11**, A, and Text-fig. 4.

By what steps this elongated ridge lying behind each coelomic septum develops into an adult nephridium I have shown in fig. 11 (A–F). The earlier stages, in which the nephroblasts and their derivatives multiply, form masses of cells at septal places which segregate further into two groups, a smaller one, the cells of which push their way into the intersegmental septa, and a larger one, the cells of which move backwards and form the so-called 'retroperitoneal' group of cells, which forms the elongated solid ridge bulging into the coelomic cavity, can all be followed in a few series of longitudinal and transverse sections; but once the nephridial rudiment has reached the size and shape of the elongated mass, shown in fig. 11 A, we can follow its further development best in whole embryos that have been opened in the mid-dorsal line, their endoderm with food-yolk removed, and the remaining portion mounted flat.

The ridge-shaped mass of nephridial cells grows in the middle, and we soon get a sort of papilla-like protuberance ; this papilla elongates further into a long loop, having its two limbs close together. At this stage, while the two ends of the loop forming the proximal part are attached to the body-wall, the loop itself forming the distal part lies free in the coelomic cavity (fig. 11 c). This loop now elongates further, and, side by side with the elongation of the loop, we find its two limbs getting more and more closely pressed together so as to form one compact lobe. A bend appears, at this stage, towards the base of this lobe, and we now get two more or less distinct divisions of the embryonic nephridium, the one distal to the bend (fig. 11 D) and lying free, and the other proximal to the bend and connected with the body-wall. The distal portion is now a compact structure and goes to form the short straight lobe of the adult nephridium. Although it has visibly lost its double character, we must note that it is really double morphologically, having been formed by a close apposition of the two limbs of the loop.

The proximal portion of the developing nephridium, the part connecting the bend with the body-wall, is now the seat of further growth. In this portion the double nature of the nephridial loop persists for a time (fig. 11 D), but, soon after, the two limbs of the loop at its proximal end, which are really the two opposite ends of the original ridge, come closer together and elongate further. This further elongation of the part proximal to the bend results in the formation of a twist, a little way from the bend, resulting in a condition of the nephridium represented in fig. 11 E. Elongation and twisting go on further until we get the long twisted loop fully formed, with the number of twists characteristic of the adult nephridium. The straight lobe and the twisted loop having been fully formed, the proximal end connecting the nephridium with the body-wall becomes narrow and slender and forms the terminal duct of the nephridium. This duct has meanwhile grown through the thickness of the body-wall, and opens to the exterior in front of the row of setae occurring in the middle line of each segment.

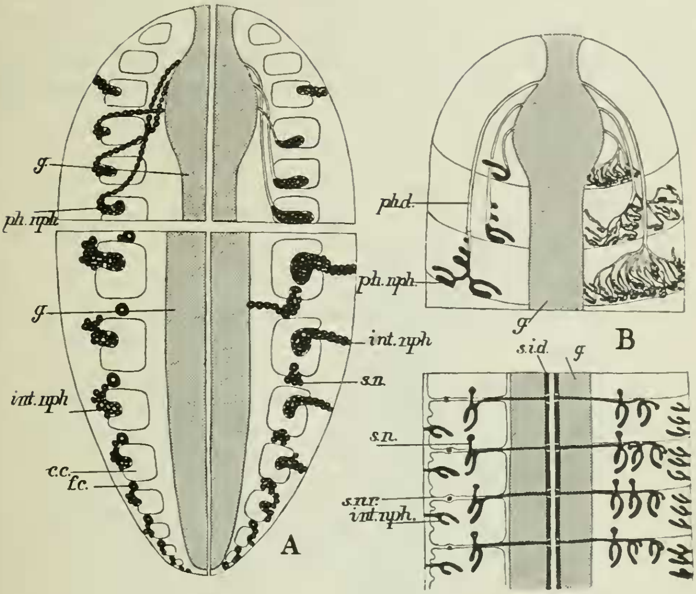
In this fully-formed integumentary nephridium we have to note the absence of either a coelomic funnel or a solenocyte or ' flame-cell '. During the course of development, when the two ends of the elongated nephridial ridge come close together, one end develops into the terminal duct opening to the exterior, while the other remains blind and does not develop any structure at all. These nephridia develop an intra-cellular canal and cilia like the septal ones ; and, no doubt, the excretion in their case takes place by means of the diffusion of the coelomic fluid through their permeable walls.

6. Development of the Primary Septal Nephridia.

When the embryo has acquired a pair of integumentary nephridia in each segment—fully developed in the anterior and in various stages of development in the posterior segments —the second set of nephridia, i.e. the septal, make their appearance. These form the second pair of nephridia in the body segments of Pheretima (Text-fig. 1 B). The first fifteen segments of the embryo do not develop this second set of nephridia, which appear only in segments behind the first fifteen. Unlike the primary integumentary nephridia the septal primary nephridia appear on both sides of the dorsal vessel instead of the nerve-cord. They form two rows, one on each side of the dorsal vessel, at a distance of about 160 μ from it in an embryo 9 mm. in length. The alternate or scattered arrangement characteristic of the integumentary nephridia of the first stage does not obtain in these septal ones, which occur in two straight rows.

These nephridia of the second set have no connexion with the body-wall, but appear from a very early stage, as their name implies, as outgrowths on the intersegmental septa. They can then be first recognized in whole mounts as masses of cells on the septum, projecting on its posterior surface. These nephridial masses on the septa can be recognized with certainty at the earliest in embryos, about 8 to 9 mm. in length, which have been opened in the mid-ventral line, their yolk removed and the rest including the endoderm mounted flat.

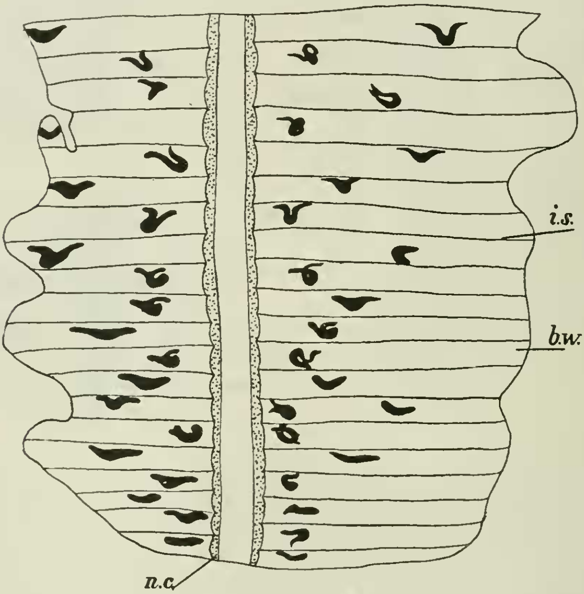
Text-fig. 3.



A series of three diagrams showing the common origin and development of the three types of nephridia in Pheretima embryos. In *A* (left half) the integumentary nephridia are seen pushing themselves into the coelomic chambers while the 'funnel-cells' are travelling dorsalwards between the adjoining coelomic chambers. The origin of the pharyngeal nephridia and ducts is also shown. The right half shows the nephridia at a more advanced stage of development. Septal nephridia are developing between the adjoining peritoneal sheets, while the ducts of the pharyngeal nephridia are formed even before the nephridia themselves are fully formed. In *B* (on the left) is shown the development of secondary nephridia, while the right half shows more or less the adult condition of nephridia in the worm. *n.t.*, nephridial teloblasts; *nph.c.*, cells of the nephridial row; *c.c.*, coelomic cavities; *int.nph.*, integumentary nephridia; *s.n.*, septal nephridia; *s.n.r.*, rudiment of a septal nephridia; *ph.nph.*, pharyngeal nephridium; *ph.d.*, duct of pharyngeal nephridia; *g.*, gut; *s.i.d.*, supra-intestinal excretory duct; *f.c.*, funnel-cells.

In such preparations of embryos (Text-fig. 5, part of an embryo about 9 mm. long) we can follow these nephridial rudiments antero-posteriorly. The dorsal vessel in embryos of this age is double in the posterior portion, and consists of two lateral vessels lying on the sides of the gut. Anteriorly,

Text-fig. 4.

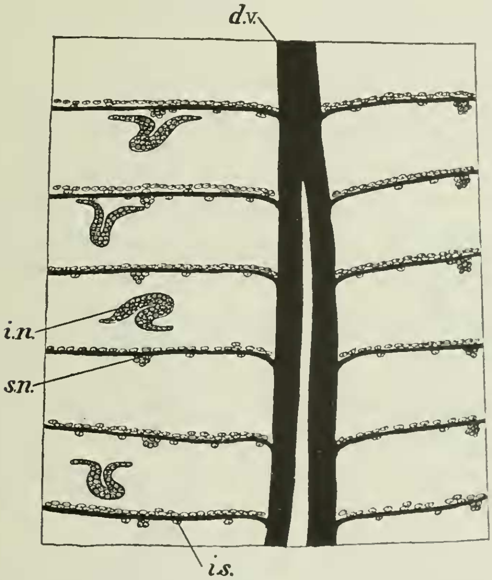


Portion of an embryo mounted flat after removal of the gut, showing the relative position of the developing integumentary nephridia in successive segments. *b.w.*, body-wall; *i.s.*, intersegmental septa; *n.c.*, nerve-cord.

however, the two dorsal vessels converge and fuse to form one single vessel, and we find it as such in the mid-dorsal line in the anterior part of the embryo. Following the nephridial masses from the septum 15/16, we can trace them backwards a good way beyond the point where the two converging vessels meet to form the single dorsal vessel (Text-fig. 5). The further development of these septal nephridia can be followed in whole mounts of embryos, 9 to 18 mm. in length, flattened after being opened from the ventral and not the dorsal side.

As the nephridial mass grows in size we can soon distinguish the two ends of the growing nephridium, as shown in Text-fig. 6. One end grows inwards along the septum towards the dorsal vessel, beneath which it meets its fellow of the other

Text-fig. 5.



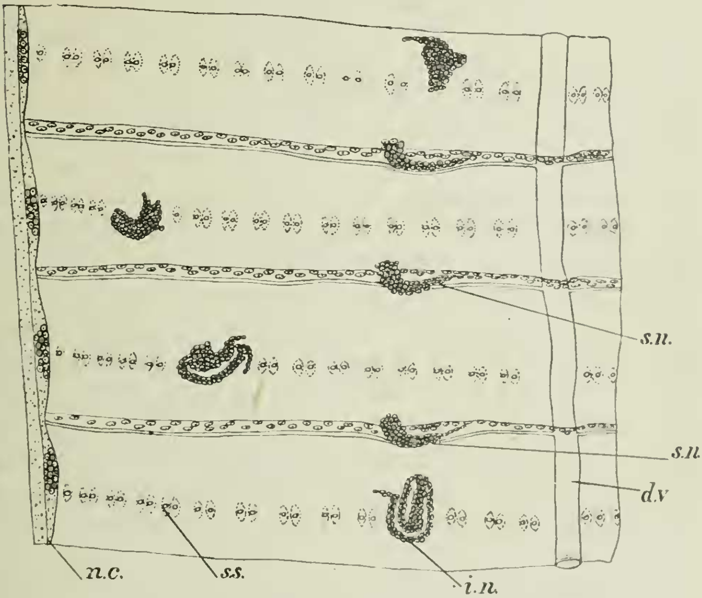
Portion of the whole mount of an embryo 8 mm. in length, showing the rudiments of the septal nephridia on each side of the dorsal blood-vessel. *d.v.*, dorsal blood-vessel, double behind; *i.n.*, integumentary nephridia; *s.n.*, septal nephridia; *i.s.*, intersegmental septa.

side to form the supra-intestinal excretory duct; we can call this end the centripetal end. The other end of the nephridial rudiment at this stage is away from the dorsal vessel, and proliferates to form a mass of cells which project in front of the septum to form the beginnings of the 'funnel' of the nephridium. This pre-septal portion of the nephridium soon attains to a considerable size, and is a prominent feature of the septal nephridia at all stages of their development (fig. 12). While these two ends of the nephridium—the ‘ centripetal ’ end and the ‘ funnel ’ end—are growing and differentiating, the portion of the nephridial mass between the two ends also grows and forms a papilla-like projection behind the septum (fig. 12 B). This papilla elongates to form a loop, the two limbs of which come close together ; a bend appears, and the portion distal to the bend forms the rudiment of the short straight lobe of the adult nephridium. This stage of the development of the septal nephridium is represented in fig. 12 C. The two ends of the nephridium are attached to the septum while the body of the nephridium, consisting of a newly-formed straight lobe (*s.l.*), distal to the bend, and a growing region proximal to the bend, between the latter and the attached ends of the nephridium, lies free in the coelomic cavity. As this growing region elongates (fig. 12 D) the two limbs come close together, and, as a result of elongation, twists appear, which grow to form the spirally twisted loop of the adult nephridium (fig. 12 and Text-fig. 7). We thus get the main body of the nephridium, consisting of the short straight lobe and the long spirally twisted loop, fully formed. Histological differentiation, along with the formation of intracellular canals with cilia lining them at intervals, completes the development of a nephridium.

As will be seen by comparing the foregoing account of the development of a septal nephridium with that of an integumentary nephridium described in the last section, the successive steps of growth in the two cases are very similar if not identical. The chief difference lies, of course, in the fate of the two ends of the nephridium. In the case of a septal nephridium one end grows out to be pre-septal and is differentiated to form the ‘ funnel ’, the other end forms the terminal duct which runs along the septum, parallel to the commissural vessel, and joins its fellow to form the supra-intestinal duct ; on the other hand, in the integumentary nephridium the ‘ funnel ’ end is blind, and the terminal duct opens on the surface of the skin.

We have now followed the development of a septal nephridium from a stage when it consists of a mass of cells on the septum (fig. 12 A) to a stage when it has attained to its adult structure (Text-fig. 7). But in order to assign these septal nephridia to one of the three primary germ-layers we must trace the ultimate origin of this septal mass—the unformed septal nephridium. We must note that an intersegmental

TEXT-FIG. 6.



Portion of the whole mount of an embryo showing septal nephridia at a more advanced stage of development than those in fig. 13. *s.s.*, setal sacs ; other letters as above.

septum is morphologically double and results from a coalescence of the two layers of peritoneum covering the two faces of the septum, and that although this double character of a septum is not discernible in sections of an adult worm the two layers of peritoneum can easily be distinguished in longitudinal sections of embryos. The question naturally arises as to whether this nephridial mass arises by a proliferation of one or more cells belonging to the two layers of peritoneum forming the septum.

like the testes and ovaries, or whether the mass arises by multiplication and growth of one or more cells lying between the two adjoining sheets of a septum. Is the septal nephridium intra-peritoneal or inter-peritoneal ; or, in other words, is it mesodermal or ectodermal ? This is the fundamental morphological question to be answered.

We have already noticed that during the course of development of the primary integumentary nephridia the mass of nephridial cells lying opposite and behind the intersegmental septa, underneath the coelomic epithelium, segregates early on into two groups—one forming the 'retroperitoneal' group of cells and developing into an integumentary nephridium, and the other consisting of a few cells that push their way into the septum between its two layers of peritoneum. This second group, which is directly traceable to the original nephric row and has thus the same source as the integumentary nephridia, is in fact the primordial rudiment of the septal nephridia.

In a series of longitudinal sections of an embryo about 6 mm. long, we can trace how a cell from this primordial group travels through the septum to take up its final position in the row of septal nephridia on each side of the dorsal vessel. If we examine, in this series, a septal nephridium on one of the anterior septa—say the twentieth—we find that it lies at a little distance from the dorsal vessel immediately internal to the commissural vessel (fig. 14 c). This incipient nephridium and the commissural vessel are both situated between the two sheets of the septum, one below the other (fig. 14). As we trace this nephridial rudiment backwards we find that it retains the same relative position with regard both to the dorsal and the commissural vessels. We can thus trace the nephridial rudiment, consisting of a few cells as it lies dorsally on each side of the dorsal vessel on one of the anterior septa, back through successive segments to the posterior end of the worm, where the nephridial rudiment lies ventrally on each side of the nerve-cord, and is just beginning to push its way into the edge of a septum. On examining the sections shown in figs. 13 and 14 two fundamentally important facts come out.

The first is the inter-peritoneal situation of the rudiment of a septal nephridium, i. e. in other words, the septal nephridia are not derived from one of the cells belonging to the peritoneal lining of the septa but take their origin from cells lying between the two sheets of peritoneum forming a septum. The second is that these rudiments can be traced directly to the original nephric row. Since the original nephric row is ectodermal in origin we have established the ectodermal origin, in the last analysis, of the septal nephridia.

In all descriptions of previous work on the development of nephridia in earthworms, mention is made of a 'funnel-cell' ('Trichterzelle'), a term which is used in at least two senses. It is either used for a single large cell which is separated off very early from the nephric row and forms the forecast of the whole nephridium, or it is used for the most internal cell of a series or group of cells which go to form the whole nephridium. and, in this case, the 'funnel-cell' gives rise only to the funnel of the nephridium. The term is used in the former sense by Bergh in the case of Criodrilus and Lumbricus (**6** and **7**), and in the latter sense by Staff in the case of Criodrilus (**15**).

During the development of nephridia in Pheretima also we can distinguish a large cell which is probably the equivalent of the 'funnel-cell'. So far as the development of the integumentary nephridia are concerned, the 'retroperitoneal' group of cells, which give rise to them, contains no 'funnel-cell' in it, nor, as we have seen, do we get a funnel formed in the adult integumentary nephridia. But with regard to the septal nephridia we can distinguish a cell larger than others at almost all stages of their development. In fig. 6, which represents part of a transverse section of the posterior end of a young embryo, we can distinguish one large cell in connexion with the septum on each side. Further, during the passage of this cell to its dorso-lateral position, one cell can always be distinguished by its very large size as compared with the surrounding peritoneal cells. Finally, when the rudiment of the septal nephridium consist of a group of three or four cells lying on the septum on each side of the dorsal vessel, one of the cells of this group is larger than the rest (fig. 14 B), and we can infer that this large cell is the so-called 'funnel-cell'. It would seem, therefore, that this large cell, as it pushes itself into the septum, is the forecast of the whole nephridium, and is a 'funnel-cell' in the sense in which Bergh uses it. And later this large cell divides and gives off cells smaller in size than itself; and while these smaller cells go to form the body of the nephridium the large cell develops into the funnel and becomes a 'funnel-cell' in the sense in which Staff uses it.

7. Development of the Secondary Nephridia, Septal and Integumentary.

At the end of the second stage of nephridial development, as we have seen, a typical segment of the embryo contains two pairs of nephridia—an integumentary and a septal. Soon after, rudiments of other nephridia, both septal and integumentary, begin to appear. These rudiments of secondary nephridia (all nephridia appearing after the first pair, septal and integumentary, have been grouped together under the term 'secondary') can be seen both in sections and in whole preparations of embryos of suitable age as deeply-staining masses of cells on the septa and the body-wall. In order to study the development of these secondary nephridia, two sets of embryos should be selected—the first set, consisting of those embryos which are fully formed and are about to hatch out of their cocoons: these show the secondary nephridia at a fair degree of development; the second set, consisting of those embryos which are not fully formed and would take some time before they are ready to hatch out: these show secondary nephridia in their very early rudimentary condition. It may be difficult, in the beginning, to distinguish embryos belonging to these two sets, but when one gets familiar with them after opening a number of cocoons, one can always distinguish them with a fair degree of accuracy. A surer method of distinguishing the embryos of two sets externally is to examine the setal line. In fully-formed embryos the circlets of setae are complete, and, on examination of the embryo under low power, we can see the setae; but in younger embryos, although setal sacs and muscles can be made out in sections, the setae are not yet formed and so cannot be distinguished externally.

(*a*) Secondary Septal Nephridia.

The secondary septal nephridia arise very much in the same way as the primary septal pair. They appear ventral to the primary septals and, like the latter, appear in pairs. It is very difficult to say whether this paired origin is maintained throughout the development of all the septal nephridia, but it is certain that the first two secondary nephridia arise in pairs. We may also note that these nephridia appear later than the secondary integumentary nephridia, since in the posterior segments of embryos with well-developed secondary nephridia in their anterior portion, through we can make out the rudiments of secondary integumentary nephridia, the septal ones have not yet been formed.

The group of cells forming a very early rudiment lies, as in the case of the primary pair, between the two peritoneal sheets of a septum and is consequently 'inter-peritoneal'. One of the cells of this group is larger than the rest and corresponds, in all probability, to the 'funnel-cell'. As regards the original relations of this secondary pair we have to note, in the first place, that their rudiments lie ventral to and at some little distance from the primary nephridia, and, secondly, that until the nephridium is almost fully formed and has developed its long terminal duct there is no connexion between this and the primary nephridium, nor are there any stray cells lying on the septum between these two nephridia. The obvious inference is that the secondary nephridia do not arise by a process of budding or the like from the primary nephridia, but do so de novo at their place of origin. Whence do the rudiments of these nephridia come?

In describing the ultimate origin of the primary septal nephridia we traced their beginnings to a group of cells which pushed their way into each septum, and which, in their turn, could be traced further to the original nephric row derived from the ectoderm. This group of cells pushing its way into the septum forms the primitive material foundation of all the septal nephridia. The primary pair is formed from one of the cells of this group, travelling dorsally on each side. More cells move into the septa and give rise to the other nephridia (secondary septals). That this does actually happen is shown firstly by the fact that there are always a number of cells lying into the septum at its junction with the body-wall, even after the rudiments of the primary pair of nephridia are well formed dorsally ; and, secondly, by the fact that we very often come across cells lying interperitoneally within the septa at a little distance dorsal and inwards to the group of cells referred to above (the group pushing its way into the septum), these cells having apparently been detached from the fundamental group and being on their way to their final place of settlement and growth.

We thus conclude that although the secondary septal nephridia do not originate as buds from the primary ones and are completely independent of them as regards their origin, they can be traced to the same source as the primary nephridia, i. e. the intersegmental group of nephridial cells, which form a store-house, giving origin to the rudiments of all the septal nephridia, primary as well as secondary.

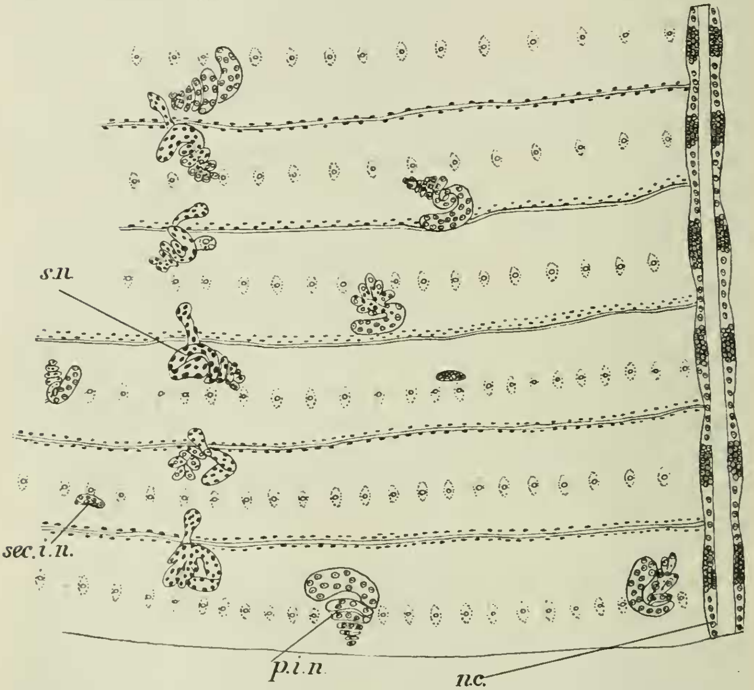
Coming now to the later development of the secondary nephridia, the chief point of interest is the topographical position of the funnel. The usual position of the funnel is always pre-septal, and we have seen that it is so with regard to the funnels of the primary pair of septal nephridia. But in the adult Pheretima (1) I have described the funnel as lying in the same segment as the rest of the nephridium, and there was thus an incongruity between the two facts of structure. This led me to a close examination of the funnels of the developing nephridia in the embryos, and also to a re-examination of the position of the funnel in the adult worm. While, on the one hand, it came out that all the primary nephridia have a pre-septal funnel, in the secondary nephridia, on the other hand, both conditions prevail—the funnel is pre-septal in some cases and post-septal in others. Both conditions are represented in fig. 15 A and B. In the adult Pheretima it was found that while a large majority of nephridia have their funnels in the same segment there are some with pre-septal funnels. The statement that all the septal nephridia have funnels in their own segments is therefore not quite universally true, as I thought before. We may note, however, that in the case of those nephridia which have the funnel in the same segment, all that happens is that the 'funnel-cell' and the cells going to form the body of the nephridium project in the same direction, either pre-septal or post-septal.

When the first secondary nephridium is fully formed, its terminal duct running along the septum meets that of the primary nephridium dorsal to it, and, similarly, the ducts of all the succeeding nephridia join those of the preceding ones, and that is how we get the formation of the septal excretory canal running parallel and internal to the commissural vessel (dorso-sous-nervien, 2). We may note that, like the septal nephridia themselves, the septal excretory canal is also inter-peritoneal.

(*b*) Secondary Integumentary Nephridia.

The secondary integumentary nephridia, in their early rudimentary condition, can be seen in older embryos about to hatch out of the cocoon. In whole mounts, as shown in Text-fig. 7, they can be distinguished as small solid deeply-staining masses on the side of and between the setal sacs. The setal sacs at this stage have not yet developed full-grown setae in them, and rudiments of secondary nephridia in sections (fig. 17) can be made out lying beneath the coelomic epithelium between the inner ends of two adjoining setal sacs. They arise at almost any place on the body-wall, like the primary nephridia ; in some segments they are found near the dorsal vessel, in others on each side of the nerve-cord (fig. 17). Whether the secondary nephridia develop from some of the nephridial cells, lying beneath the somatic peritoneum, that have been left over from the original nephridial masses, or whether they arise from epidermal cells that become nephridial at the time and migrate inwards, I cannot be sure. On

Text-fig. 7.



Portion of a whole mount of an embryo about to hatch out of the cocoon, showing the fully-formed primary septal nephridia and the primary and secondary integumentary nephridia. *s.n.*, septal nephridia; *p.i.n.*, primary integumentary nephridia; *sec.i.n.*, secondary integumentary nephridia; *n.c.*, nerve-cord.

examining Text-fig. 4 it would seem as if the nephridial substance (cells potentially nephridial) is spread over the whole of the body-wall, and any part of it might become active and form a nephridium, and hence the appearance of nephridia at all sorts of places on the body-wall. If that be the case, and if, as shown in fig. 8, nephridial cells extend on each side of the definite nephridial rudiments, we probably get these secondary nephridia formed from the stray nephridial cells lying beneath the peritoneal membrane of the body-wall. But, as in fig. 17, we have to account for a string of cells, which is not always seen, running from the nephridial rudiment to the ectoderm. It may be that it is a secondary formation leading from the nephridial rudiment to form the terminal duct.

8. Development of the Pharyngeal Nephridia and their Ducts.

The development of the pharyngeal nephridia of the fourth, fifth, and sixth segments can be followed in all its stages in the same embryos which show the development of the integumentary and septal nephridia. The pharyngeal nephridia appear at the same time as the primary integumentary nephridia, but are rather slower in growth than the latter. At a stage of development when the embryo is 5 to 6 mm. long and the integumentary nephridia of some of the segments behind the first six (e. g. seventh, eighth, and ninth) are almost fully formed and have developed their intra-cellular canals, the pharyngeal nephridia are seen as deeply-staining compact masses of cells lying on the body-wall, a pair in each segment, one on each side of the nerve-cord. They develop from the same source as the primary integumentary nephridia, i. e. from the nephridial cells belonging to the original ectodermal nephric row ; but their manner of development is different from the other two types. While in the case of the integumentary nephridia the terminal duct is very short and appears rather late in development, forming a lumen at the same time with the rest of the nephridium, the ducts of the pharyngeal nephridia develop very early. In an embryo 5 mm. in length the nephridia of the fourth, fifth, and sixth segments are small club-shaped solid masses produced into long solid strings of cells leading anteriorly to the lateral walls of the pharynx (fig. 18). The terminal ducts are thus formed earlier than the bodies of the nephridia themselves. This is still more marked at a later stage in an older embryo in which the ducts of the pharyngeal nephridia are seen to have acquired a lumen, while the nephridia themselves have not yet developed their adult form and are still solid. These ducts are intracellular, but are surrounded by the muscular tissue of the strands passing from the pharynx to the body-wall for part of their length.

The usual order of antero-posterior development is reversed in the case of pharyngeal nephridia. In the two stages of development mentioned above, the nephridia of the sixth segment are advanced further in development than those of the fifth, and the latter than those of the fourth segment.

There is only one pair of nephridia anterior to the pharyngeal ones, i. e. the one belonging to the third segment, the first two segments of the embryo being anephrous. This most anterior pair, although integumentary in character, follows the pharyngeals in their time and rate of development.

The ducts in these early stages are very thin with intracellular lumen, and are therefore to be looked upon as the elongated terminal ducts of the primary pairs of pharyngeal nephridia rather than as outgrowths from the walls of the pharynx. Three successive thickenings on the lateral pharyngeal wall mark the places of entrance of the ducts into the pharyngeal lumen. It is a remarkable fact that not only do the terminal ducts acquire a lumen before the formation of the canals in the nephridia themselves, but that they also open into the cavity of the pharynx long before the nephridia are able to function at all.

The pharyngeal nephridia, like the integumentary ones, do not develop a 'funnel', but we have to note that at an early stage when the terminal ducts have been formed and the nephridia are developing their adult structure, the pharyngeal pair come into connexion with the intersegmental septa *not in front of* but behind them. The dorsal blood-vessel and the lateral oesophageals act as the afferent and the efferent vessels to these nephridia, and the branches of these vessels near their points of origin and entrance into the main vessels lie on the septal supports.

Secondary pharyngeal nephridia arise in a way different from that of the secondary septal and integumentary ones. They do not appear independently of the primary pair but develop as buds on the nephridial ends of the pharyngeal ducts. In fig. 19 are seen three buds in the fifth and two in the sixth segment ; while in the fourth the primary nephridium itself is not fully formed yet. As these buds develop into fully-formed nephridia, their terminal ducts, longer than those of the other types of nephridia, remain continuous with the primary pharyngeal duct, or rather open into it. Thus we get a large number of pharyngeal nephridia forming big tufts and having their terminal ducts opening into these primary ducts. The primary ducts themselves, although originally very narrow and intra-cellular, enlarge and acquire a muscular investment which makes their walls thick and tough as they are in the adult condition.

9. Comparison with the Development of 'Meganephric' and the so-called 'Plectonephric' Types of Nephridia.

I have referred in brief to the known facts of development of these two types of nephridia in the historical part of this paper. So far as the 'meganephric' type of nephridia are concerned, we can compare them only with the primary integumentary nephridia of Pheretima, a pair in each segment. The obvious differences between the meganephridia of Lumbricus and the primary pair of integumentary nephridia in an embryo of Pheretima are the larger size of the former and the presence of a 'funnel' in them. In his recent memoir on the development of nephridia in Criodrilus, as already mentioned on p. 53 (**15**), Staff derives the nephridium from the 'funnel-cell' and the retroperitoneal group of cells behind each septum, both being ultimately derived from the nephridial string of cells between the ectoderm and the mesoderm. In Pheretima, as we have seen, the primary integumentary nephridia develop from the 'retroperitoneal' cells alone and there is no 'funnel-cell' taking part in their formation, and that is why they do not develop any funnel at all. The septal nephridia, on the other hand, develop from the intersegmental nephridial mass of cells which possesses a 'funnel-cell', and so we get septal nephridia with funnels developing from this source.

This meganephric, or rather the paired condition in the embryo, is superseded by, and is assimilated into, the adult condition which is 'enteronephric', so far as the septal and pharyngeal nephridia are concerned; but is 'micronephric' and diffuse so far as the integumentary nephridia are concerned. It cannot be called 'plectonephric'. If the place of opening of the nephridia be taken into consideration we can divide the nephridia into two groups: those that open to the outside on the surface of the body-wall and may be termed 'dermonephric' or 'exonephric', and those that open into any part of the gut and may be called 'enteronephric'. The former category will include the ordinary 'meganephridia' of Lumbricus, as well as the integumentary micronephridia of Pheretima and the plecto-nephridia of other worms. The latter term, i.e. enteronephric, will comprise the septal and pharyngeal nephridia of Pheretima, the 'pharyngeal tufts', the 'peptonephridia' or 'salivary glands' of such forms as Megascolides, Periscolex, and Enchytraeids, and the anal nephridia of Octochaetus and Allolobophora antipae (**14**).

Comparing the nephridial development of Pheretima with that of the plectonephridia of Megascolides (**17**) and Mahbenus (**8**), we have to note that, while in the latter the whole system results from the breaking up and branching of the first pair of nephridia, in Pheretima there is no such branching and breaking up, and all the nephridia, septal and integumentary, appear independently of one another. Moreover, the first pair of nephridia in Megascolides and Mahbenus have 'funnels' which persist in Megascolides but degenerate in Mahbenus, while Pheretima has no 'funnels' even on its initial pair of integumentary nephridia.

In my previous paper (**1**), from a study of the structure of adult nephridia, I came to the conclusion that each nephridium is a separate and discrete structure, that there is no network of any kind connecting one nephridium with the other, and that it is a mistake to describe the nephridial system of Pheretima as a ' coelomic network ' or as ' plectonephric ', implying the idea of a reticular connexion between the nephridia. This conclusion is now confirmed by the evidence we have from the embryology of the excretory system of this worm. We now know that each nephridium, integumentary or septal, originates independently of the others, and, therefore, even the embryonic connexion found in Megascolides and Mahbenus is wanting in this worm.

10. Phylogeny of the Oligochaete Nephridial System.

Before any developmental facts were known with regard to the excretory organs of Oligochaetes, it was commonly held ' that the paired nephridia (meganephridia) of most Oligochaeta were formed by reduction from a network such as now exists in Perichaeta and many other genera ' (**4**). But after the embryology of the excretory system in Octochaetus and Megascolides had been elucidated, and it was found that a meganephridial condition preceded the diffuse condition, this view had to be given up. In speaking of the phylogeny of the system, Beddard (**4**) says that ' it does not follow that the diffuse nephridia are the outcome of a branching and specialization of the paired nephridia ; what the developmental facts prove is that both paired and diffuse nephridia are formed out of similar pronephridia ; that in fact both kinds of excretory organs are equally ancient '. This view was definitely formulated by Vejdovsky (**16**), who says, ' Es hat daher meiner Ansicht nach sowohl das " Plecto- als Meganephridium " gleiche genetische Bedeutung. Beiden muss ein einfacher, paarig in jedem Segmente sich anlegender Strang—das Pronephridium—vorausgehen, aus welchem erst secundär seitliche Wucherungen entstehen, die sich als zahlreichere oder spärlichere Nephridiallappen erweisen. In grosser Menge bilden sich offenbar die Läppchen bei den mit "Plectonephridien" versehenen Oligochaeten und in den vorderen Segmenten von Megascolides. In den hinteren Segmenten des genannten Riesenregenwurmes reduciren sich die Läppchen an einige grössere, welche der Lage nach den Schlingen am Nephridium von Lumbricus entsprechen.' Vejdovsky's conclusions are based on his researches on Rhynchelmis, in which a definite pronephridial stage precedes the permanent nephridia, and on the development of nephridia in Megascolides, in which a paired pronephridial condition precedes the permanent plectonephric system.

The facts of nephridial development recorded in this paper do not lend themselves to Vejdovsky's interpretation of the evolution of the excretory system. In the first place, we cannot distinguish in the development of Pheretima a pronephridial stage as distinguished from a stage of permanent nephridia. What we do get is a paired condition in the embryo which goes to form part of the adult system and is not entirely superseded by it. Secondly, the paired nephridia themselves are not transformed, as they are in Megascolides, into the diffuse system of the adult, but numerous nephridia arise independently to be added to the primary integumentary nephridia. In the third place, the adult Pheretima does not show the condition referred to by Vejdovsky in some other worms ('Riesenregenwurmes'), where the anterior segments have numerous nephridia but the posterior ones show the paired meganephric condition.

Since the diffuse and paired forms of the excretory system occur in genera which are so nearly related, Beddard (3) thinks there can be no profound gap between the two kinds of organs. But when one takes into account the fact that in the family Perichaetidae, Pleionogaster possesses nephridia of the diffuse type all opening to the exterior, Megascolex has a pair of large nephridia in each segment in addition to the small scattered nephridia, while Perionyx and Diporochaeta have only large paired nephridia, it becomes very difficult to think of and offer an explanation for the intermediate evolutionary stages between the condition in Perionyx (exonephric) and that in Pheretima (enteronephric). Although we can derive the diffuse condition of Pleinogaster from Perionyx, through such forms as Megascolex showing an intermediate condition, we cannot ignore the fact that the gap between the 'exonephric' (p. 86) and 'enteronephric' conditions is very deep indeed.

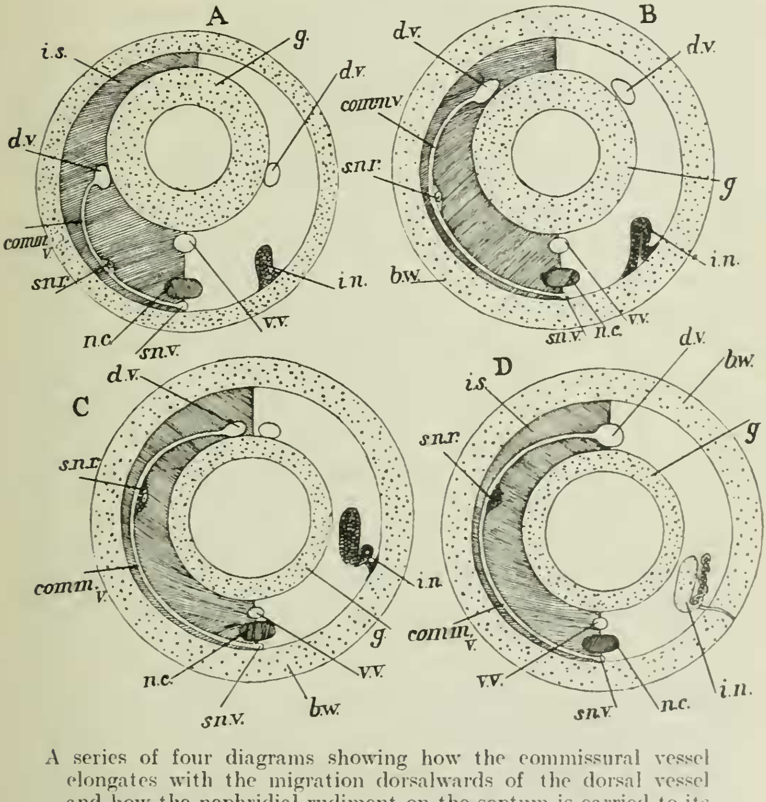
A few facts in the embryology of the nephridial system, however, seem to throw some light on the possible evolution of the enteronephric system. I have already shown (p. 67) that the primary integumentary nephridia have a common source of origin in the nephridial masses lying opposite the intersegmental septa. We have also seen that, while the integumentary nephridium has no 'funnel-cell', it is represented in the rudiment of each septal nephridium, and consequently the former lacks and the latter possesses a 'funnel' in the adult condition. It is possible to suppose that the first great step in the process of evolution of the enteronephric system was the severance of the connexion between the 'funnel' and the 'body' of the nephridium. That this severance has probably taken place in Pheretima is supported by very strong evidence from the embryology of the nephridia of Octochaetus (**3**), Megascolides (**17**), and Mahbenus (**8**). In all these forms there is a paired meganephric condition in the embryo, and each nephridium is provided with a well-developed funnel. In the transformation of this embryonic into the adult condition the part to degenerate first is always the duct following the funnel. In Octochaetus (**3**), Beddard found that the change took place by the disappearance of the lumen in the portion nearest the funnel. Vejdovsky (**16**) has found in Megascolides that the paired embryonic nephridia have a funnel from which leads a straight duct without lumen, and that this duct joins the nephridial loops. During development the connecting part of the original tube (i. e. the straight solid duct) first degenerates into a mere strand of connective tissue and finally breaks up entirely. But the funnel remains and forms part of the large nephridium in the adult. Bourne (**8**) found a similar condition in Mahbenus, the funnel, however, in this case degenerating entirely.

It is easy to derive the condition in Pheretima from what takes place in Megascolides. In the latter the funnel, with part of the tube following it, develops into the large paired nephridium with funnel, a pair to each segment, while the body of the nephridium gives rise to a network of minute excretory tubules. In Pheretima the separation of the funnel from the body of the nephridium is carried a little farther and takes place early in ontogeny. The result is essentially the same as in Megascolides, i. e. the formation of two kinds of nephridia: in this case the larger septal nephridia with funnels and the smaller integumentary ones without funnels. The evolution has taken place along the same lines in Octochaetus and Mahbenus also, but in these two genera the degeneration and disappearance of the portion following the funnel has likewise affected the funnel which also degenerates, and that is why we get only one type of nephridium without funnel in these two cases, although Beddard found some nephridia with funnels towards the posterior end of Octochaetus, along with those without funnels.

In Pheretima the 'funnel-cell' along with some other nephridial cells separate off early from the main nephridial mass, and while the integumentary nephridia develop at once from the main nephridial mass the development of the septal ones from the 'funnel-cell' comes a little later. We tacitly assume, of course, that the 'funnel-cell' itself by division is capable of giving rise to the whole nephridium, and this is what actually takes place (cf. figs. 13 and 14). Why the funnel gets separated off from the main body of the nephridium during development in Octochaetus and Mahbenus, and why the separation begins so early in Pheretima—whether it took place in phylogeny before or after the septal nephridia had acquired their openings into the gut—are questions difficult to answer.

How does this 'funnel-cell' travel dorsalwards and take up a dorso-lateral instead of its usual ventral position? That it travels dorsalwards can be seen easily from figs. 13 and 14, and that this is very unusual can be realized from the fact that

Text-fig. 8.



A series of four diagrams showing how the commissural vessel elongates with the migration dorsalwards of the dorsal vessel and how the nephridial rudiment on the septum is carried to its dorso-lateral position. *d.v.*, dorsal vessel (double in *A*, *B*, and *C*); *v.v.*, ventral vessel; *n.c.*, nerve-cord; *sn.v.*, subneural vessel; *i.s.*, intersegmental septum; *i.n.*, integumentary nephridium; *s.n.r.*, rudiment of a septal nephridium; *b.w.*, body-wall.

ordinarily the funnel in a nephridium is the most ventral part of it and lies almost next to the ventral nerve-cord. But although away from the nerve-cord, the position of the primary septal nephridia is not very unusual if we bear in mind the scattered arrangement of the integumentary nephridia (Text-fig. 3). By comparing the position of the septal and integumentary nephridia in Text-figs. 5 and 6 the discrepancy does not seem to come to very much. But that there is a shifting dorsalwards, however little (and in many septa a great deal), admits of no doubt. This can be explained, however, easily, if we take into account the facts of development and growth of the septa and the structures connected with them, e. g. the commissural vessel and the dorsal vessel. In the series of diagrams in Text-fig. 8 I have tried to illustrate the gradual growth and formation of the commissural which connects the subneural and dorsal vessels. The dorsal vessel is formed by the progressive backward concrescence of the two lateral vessels, which, as a rule, lie at the dorsal edge of the advancing mesoderm bands. The commissural, which appears very early and connects the subneural with the lateral (the semi-dorsal so to speak), lies from the very beginning between the two sheets of the mesodermal septa. From an examination of figs. 13 and 14 it becomes clear that the ‘ funnel-cell ’ or the rudiment of the septal nephridium is closely associated with the commissural vessel, lying in the same intraseptal cavity with and ventral to the blood-vessel. It would seem that in the process of migration of the lateral vessels to the mid-dorsal position the commissural vessel must keep pace and travel dorsalwards. Further, in the general growth of this vessel dorsally, the ‘ funnel-cell ’ or nephridial rudiment closely associated with it also travels dorsalwards, and hence results the dorso-lateral position of the primary septal nephridium.

That the commissural vessel and the rudiment of the septal nephridia lie in the same hollow of the septum between the two sheets of mesoderm in the embryo is clear from figs. 13 and 14, and is evidence of the close association of these two structures. That there is some morphological relationship is also shown by the fact that, in the adult worm, the septal nephridia are only present on those septa that have a commissural vessel. In the first fourteen segments of the worm there are no septal nephridia and no commissural vessels either.

Once the 'funnel-cell' reached its dorso-lateral position, it developed into a nephridium with a funnel (a septal nephridium); but it would seem that the terminal duct of the nephridium had, so to speak, lost its original course, having been removed from the body-wall and having been caught in the 'tunnel' of the septum containing the commissural vessel. The terminal duct followed the course of the vessel and travelled towards the mid-dorsal line, where, on meeting its fellow of the other side, it formed the supra-intestinal excretory duct. It would be a case of induced development and growth, stimulated by the course and development of the commissural vessel. When we have once got a septal nephridium formed in the dorso-lateral position, its terminal duct would tend to find a way out. But since the way to the body-wall is blocked, the terminal duct lengthens out and follows the course of the commissural vessel. Examples of this kind of 'dependent differentiation', a term due to Roux, are found in the experiments of Lewis and Spemann. Lewis has shown that a lens will be formed from any patch of ectoderm taken from some other part of the body and grafted over the optic cup during the development of the eye in Amblystoma and some species of frogs. Spemann and Lewis have also found that in the absence of contact between the optic cup and the ectoderm the cornea is not developed, that is to say, the overlying ectoderm does not 'clear' (lose its pigment), it does not thin out, and Descemet's membrane is not formed.[1]

Once the supra-intestinal duct is formed in the mid-dorsal line above gut, the only possible way to discharge the excretory fluid is to have communications with the gut in each segment. This tendency of the nephridial ducts to open into the gut has been noticed in other worms also. Rosa (**14**) found in one species of Allolobophora (A. antipae) that all the nephridia of the posterior region of the body, instead of opening on the exterior, communicate with a pair of longitudinal canals which open posteriorly into a median vesicle communicating with the rectum. It would appear that in outline

[1] Jenkinson's 'Experimental Embryology', pp. 271-7.

the condition of nephridia in the posterior region of the body of Allolobophora antipae is remarkably similar to that in the whole length of the body of Pheretima. On the formation of communications between the supra-intestinal ducts and the lumen of the gut, the essentials of the 'entero-nephric' system are completed.

The formation of secondary septal and integumentary nephridia is not difficult to explain. The septals are, no doubt, traceable to the same source as the primary nephridia, i.e. to the cell-group making its way into the intersegmental septum. The primary nephridium already has a pre-septal funnel, while, of the succeeding secondary ones, most have a post-septal funnel, there are some with a pre-septal funnel. It is possible that the original 'funnel-cell' has something to do with the pre-septal or post-septal position of the funnel. If the funnel is formed from the original 'funnel-cell' or a derivative of it, we get a pre-septal funnel; but if the funnel is formed from one of the other cells which takes up the character of the 'funnel-cell', a post-septal funnel results.

As regards the secondary integumental nephridia, their separation from the primary nephridia and from one another has gone much deeper and further than that shown in the developing nephridia of Megascolides (**17**) and Mahbenus (**8**). They are coeval in origin, but not connected in any part of their development.

Both the buccal cavity and the pharynx, forming that portion of the alimentary canal which lies in the first four segments of the body, belong to the stomodaeum; and since the latter is morphologically external, the pharyngeal nephridia opening into the stomodaeum may be said to open on the ectoderm. But the facts of development do not help us in understanding the possible course of evolution of these nephridia. We cannot, for example, assume that these nephridia are really integumentary, but have come to occupy their present position and relationship on account of the anterior portion of the worm being formed into an 'introvert' or a stomodaeum. This assumption does not work in giving us the adult structure from the hypothetical original condition of these nephridia. The development of secondary pharyngeal nephridia as distal buds on the pharyngeal ducts is remarkable.

11. Material and Technique.

The material for this work consisted of cocoons of Pheretima containing embryos of various ages. In the Kew Gardens, where I got my supply of these worms from, they are found along with two or three other genera in the soil of the Lily House, and, if the cocoons were collected from that soil, it would be difficult to distinguish the cocoons of one genus from another. Accordingly, to be quite sure of the specific identity of my material, I tried the isolation and culture method, which I briefly describe below.

I took common garden soil and sterilized it for two or three hours to kill all organisms in it, specially the cocoons of other worms, eggs of insects, &c. This earth was mixed with sand, and finally I added to this mixture a quantity of decaying leaves which had also been previously sterilized. Four garden pots, the bottom holes of which were closed with corks, were filled with the sterilized soil and about fifteen worms of this species of Pheretima were kept in each pot. These pots were kept in a hot-house with an average temperature of 60° F.; the earth in the pots was kept moist and sterilized decaying leaves were added from time to time. After the first two months I was able to get cocoons in this way almost throughout the year, and was always sure that the cocoons were of Pheretima alone and of no other worm.

Various methods were tried to sift out the cocoons from the earth, but the least troublesome, and therefore the best, is to put a heap of earth in a fine sieve and to stir the earth while keeping the sieve in a bucket of water. The earth passes through the sieve and settles down at the bottom of the bucket, while the cocoons are left in the sieve along with the large pebbles and pieces of stone. The cocoons can be easily found and picked up with a wet paint-brush.

Since the cocoons of Pheretima have a transparent shell, the embryo inside a cocoon can be easily seen under a binocular microscope by transmitted light. It is therefore easy to know the age and size of the embryo before opening the cocoon. Since in a given lot of earth the cocoons are of all ages, we can at once select an embryo of the desired age, provided we have a large number of cocoons. As a rule there is only one embryo in each cocoon, but we sometimes meet with two or even three.

The cocoons are opened in salt solution by means of a pair of sharp needles under the binocular microscope. Very early stages (blastulae and gastrulae) were mounted whole in clove oil after staining with paracarmine. Two pieces of hair were placed below the coverglass, which enabled the rounded embryo to be rolled under the coverslip in order that it could be examined from all sides.

Embryos of about 4 to 6 mm. in length were used both for whole mounts and sections for the study of integumentary nephridia. The embryos while in salt solution were always narcotized by ether and fixed either with Bouin's fluid or corrosive-acetic or Petrunkewitsch. The latter solutions were found preferable to Bouin, since this fluid hardens the food-yolk very much and makes it brittle for section-cutting. Serial longitudinal sections of a few embryos of suitable age enables one to follow the development of integumentary nephridia fairly completely. A series of transverse sections, 5 μ in thickness, of an embryo about 300 μ in length, was very useful in following the very early stages of development, e.g. the teloblasts and their development. For the later stages of development of primary nephridia, as represented in Text-fig. 4, embryos fixed in Bouin or corrosive are slit open by means of a sharp needle along the mid-dorsal line, the roll of albuminous material filling the gut is removed, and with a little care the endoderm itself is removed. What is left is the body-wall with the nephridia attached to it. This is stained with paracarmine, flattened out, and mounted whole.

Older embryos, about 10 mm. or more in length, are fixed in the same way and opened by a mid-ventral incision; the albumen is removed but not the endoderm: these, when mounted flat, show the septal nephridia in all stages of development, as represented in figs. 14 and 15. Since they lie in two rows, one on each side of the dorsal vessel, it is best to open the embryos from the ventral side and look for the nephridia on each side of the dorsal vessel. In order to avoid any displacement of the septal nephridia it is best to leave the endoderm on; but it is necessary to brush carefully the inside of the embryo with a fine camel-hair brush, so that all yolk-material sticking to the inside of the gut is removed and the preparation rendered quite transparent to show the septal nephridia to the best advantage.

The most difficult part of the task, however, was to trace the initial stages of development of the septal nephridia—to find out whether the ' rudiment ' ('Anlage') of the septal nephridia arose as a multiplication of one or more cells belonging to the walls of the adjoining coelomic sacs (the septa), or it was formed by a group of cells between the two contiguous sheets of the intersegmental septa. For this purpose it was necessary to have serial longitudinal sections of the dorsal and dorsolateral parts of an embryo of suitable age, i.e. one in which one could expect to find the septal nephridia in very early stages of development. The difficulty in getting such sections arises from the fact that the gut in embryos of this age is so enormously distended with food-yolk as to squeeze out of existence altogether the coelomic cavity between the body-wall and the gut dorsally. Consequently it becomes impossible to distinguish, in sections of such embryos, the septa and the nephridial masses on them. Many series of sections were cut of embryos which had been flattened out, after being cut open ventrally and the food-yolk removed. In these series, although the coelom could be distinguished in some of the segments, it was obliterated in others, and no accurate and reliable observations could be made. In many of the embryos part of the posterior end was cut off before fixation to allow the food-yolk to ooze out and thus let the wall of the gut shrink away from the body-wall, restoring the coelomic cavity on the dorsal side. I did not meet with much success even by this method. At last I was lucky in finding two embryos, one of which was just of the right age (about 6 mm.) and in both of which the gut was narrow and the coelomic cavity all round very spacious. A complete series of longitudinal sections of one of them gave me all the stages of development of the septal nephridia, and I was able to establish, firstly, that the septal nephridia develop between the two peritoneal sheets (inter-peritoneal), and, secondly, that they could be traced to their final septal position from their first place of origin in the primary nephric row in the body-wall.

12. Summary.

1. The three kinds of nephridia—integumentary, septal, and pharyngeal—appear at successive stages of development of the embryo ; the integumentary preceding the septal and pharyngeal, both of which develop simultaneously.

2. All the three kinds can be traced back to the original row of nephridial cells of ectodermal origin. Thus all the different nephridia are ultimately derived from one common origin.

3. The primary pair of integumentary nephridia are the first to appear from a ' retro-peritoneal ' group of cells. The rudiments lack the ' funnel-cell ', and consequently a ' coelomic ' funnel is never developed in these nephridia. They open to the exterior on the body-wall.

4. These primary integumentary nephridia do not appear in the same position in successive segments of the embryo, but are irregularly distributed all over the body-wall.

5. The septal primary nephridia can be traced back to a group of nephridial cells, including the ' funnel-cell ', which make their way into each septum between its two adjoining peritoneal lamellae.

6. The primary septal nephridia have always a well-developed pre-septal funnel, and appear along a straight line on both sides of the dorsal vessel. They appear after the primary

integumentary pair has reached a fairly advanced stage of development.

7. The secondary nephridia of both the integumentary and septal types are not budded off from the primary nephridia, but the rudiments of all have a common origin and separate early. They resemble the primaries in every respect, except that in the case of the septal secondaries the funnel is either pre-septal or post-septal.

8. The terminal ducts of the primary septal nephridia form the dorsal portions of the septal excretory canals on the septa, and the canals of both sides form the supra-intestinal duct on meeting the mid-dorsal line above the gut. The segmental ductules establishing a communication between the supra-intestinal duct and the lumen of the gut appear soon after the formation of the supra-intestinal ducts.

9. The primary pharyngeal nephridia of the fourth, fifth, and sixth segments develop from a 'retro-peritoneal' group of cells like the integumentary ones, and have long ducts reaching the wall of the pharynx. Secondary nephridia are formed as successive buds on the ducts, anterior to the primary nephridia.

10. The possible phylogenetic stages in the evolution of the 'enteronephric' type of nephridia are as follows: (1) the severance of the connexion between the septal funnel and the body of the nephridium; (2) migration of the severed portion, i.e. the 'funnel-cell', together with some other nephridial cells from a ventral to a lateral position in the embryo; (3) the growth of this severed portion into a septal nephridium and the acquisition by the latter of an opening into the gut; (4) the elongation of the terminal ducts of all septal nephridia towards the mid-dorsal line (induced by the course of commissural vessels) and the formation of continuous supra-intestinal ducts. It is problematic whether the severance of the connexion between the funnel and the body of the nephridium took place before or after the connexion of the nephridium with the gut.

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EXPLANATION OF PLATES 5–7.

Illustrating Dr. K. N. Bahl's paper 'On the Development of the "Enteronephric" type of Nephridial System found in Indian Earthworms of the genus Pheretima'.

Fig. 1.—Whole mount of a very young embryo ($146\mu \times 130\mu$) seen in a ventro-lateral aspect, showing the mesodermal pole-cell M.P.C., the neural and nephridial teloblasts N.N.T., and the mesoderm band *m.b.* $\times 500$.

Fig. 2.—Longitudinal section of an embryo 245μ in length, showing the relative position of the mesodermal pole-cell and one of the teloblasts. The ectodermal origin of the teloblast is clearly indicated. T. the neural teloblast; *c.c.*, coelomic cavities in the mesoderm; *arch.*, archenteric cavity; *end.*, endodermal cells with yolk-granules. $\times 500$.

Fig. 3.—Ventral portion of a transverse section from the posterior end of an embryo 300μ long, showing the origin of the teloblasts in the ectoderm. The teloblasts are still embedded in the ectodermal layer, but are sharply marked off from the definitive ectodermal cells. On the right are seen two teloblasts, the ventral one being the neural and the lateral one the nephridial. On the left are seen three cells still embedded in the ectoderm which lie in front of the teloblasts and have been budded off from them. *n.t.*, neural teloblast; *np.t.*, nephridial teloblast; *c.t.*, cells budded off from teloblasts; *m.s.*, hollow mesodermal somites with coelomic cavities, *c.c.*; *ect.*, ectodermal cell. $\times$ *cir.* 620.

Fig. 4.—Ventral portion of the section just in front of the one shown in fig. 3, showing the gradual demarcation of the neural and nephridial cells from the definitive ectoderm. *lat.t.*, lateral teloblast; *n.c.*, neural cells; *nph.c.*, nephridial cells. $\times$ *cir.* 664.

Fig. 5.—Ventral portion of a longitudinal section through the hind end of an embryo about 3 mm. long, showing the nephridial string of cells breaking into the cell-masses destined to give rise to the nephridia. M.P.C., position of mesodermal pole-cell; *nph.t.*, nephridial teloblast; *nph.l.*, nephridial layer of cells; *m.l.*, layer of mesoderm with coelomic cavities; *ect.*, ectoderm. $\times 545$.

Fig. 6.—Transverse sections of an embryo 300μ long, from which figs. 3 and 4 have been drawn, showing the 'funnel-cells' in section between the two adjoining coelomic cavities, i.e. in the septa. *n.c.*, nerve-cord; *nph.c.*, groups of nephridial cells; *f.c.*, funnel-cells; *ect.*, ectoderm. $\times 400$.

Fig. 7.—Portion of a longitudinal section of an embryo, showing the nephridial groups of cells pushing into the intersegmental septa. Other letters as before. $\times 640$.

Fig. 8.—Five consecutive longitudinal sections of a series taken from the hinder portion of an embryo about 4 mm. long, showing the development of primary integumentary nephridia, and their relations with the intersegmental septa. *nph.*, nephridia (integumentary) ; *nph.c.*, nephridial cells pushing their way into the septa ; *i.s.*, intersegmental septum ; *c.c.*, coelomic cavity ; *ect.*, ectoderm.

Fig. 9.—A portion of a longitudinal section of the same embryo as in fig. 8, showing the developing integumentary nephridia. Letters as in fig. 8. ×1,200.

Fig. 10.—Portion of a longitudinal section of an embryo, showing the semblance of a 'funnel', the only series in which a 'funnel' is seen. *f.*, pseudo-funnel. ×1,160.

Fig. 11 (A–F).—A series of diagrams showing the developing stages of an integumentary nephridium, taken from whole mounts of embryos with endoderm and albumen removed. Nephridia lie posterior to the septa. *i.s.*, intersegmental septa ; *s.l.*, short straight lobe of the nephridium ; *t.l.*, the twisted loop. × *cir.* 630.

Fig. 12 (A–E).—A series of diagrams from whole mounts showing the exact mode of development of a primary septal nephridium. *f.*, the pre-septal funnel ; *s.l.*, straight lobe ; *t.l.*, twisted loop ; *t.d.*, terminal dust ; *c.v.*, commissural vessel ; *i.s.*, intersegmental septum. ×530.

Fig. 13.—Longitudinal sections of the posterior end of an embryo about 6 mm. in length, showing how the 'funnel-cell' travels from its original position in the nephridial layer of the body-wall to its final position on the septa on each side of the dorsal vessel. 1–6 are sections of septa from the same series showing the change in position. *c.v.*, commissural vessel ; *f.c.*, funnel-cell ; *v.lat.*, ventro-lateral branches of the ventral vessel ; *c.c*, coelomic cavities. × *cir.* 1,250.

Fig. 14 (A–D).—A series of septa in longitudinal sections showing the early development of a septal nephridium ventral and close to the commissural vessel and between the two sheets of mesoderm forming a septum. *b.w.*, body-wall ; *c.v.*, commissural vessel ; *nph.r.*, nephridial rudiment. × *cir.* 1,250.

Fig. 15.—Two intersegmental septa with nephridia on them from a whole mount of an embryo about to hatch out of the cocoon, showing the developing secondary nephridia of both kinds, one with pre-septal funnel and the other with post-septal funnel. *psn.*, primary septal nephridium ; *sec.s.n.*, secondary septal nephridium with post-septal funnel ; *sec.s.n'.*, secondary septal nephridium with a pre-septal funnel. ×450.

Fig. 16.—Part of a transverse section of an embryo, showing a rudiment of a secondary septal nephridium. *c.v.*, commissural vessel ; *b.w.*, body-wall ; *n.c.*, nerve-cord ; *d.v.*, dorsal vessel ; other letters as in fig. 15. ×330.

Fig. 17.—Portions of two consecutive sections of an embryo, showing

the development of secondary integumentary nephridia. *sec.i.n.*, secondary integumentary nephridia; *ep.*, epidermis; *circ.m.*, layer of circular muscle-fibres; *long.m.*, layer of longitudinal muscles; *s.s.*, setal sacs. ×440.

Fig. 18.—Longitudinal section of the anterior portion of an embryo about 4·5 mm. in length reconstructed from serial sections, showing the early development of pharyngeal nephridia and their ducts (both being solid at this stage). ph_4, ph_5, ph_6, pharyngeal nephridia of the fourth, fifth, and sixth segments with their respective ducts; *phx.*, pharynx; $i.g._7$ integumentary nephridia of the seventh segment; *b.c.*, buccal cavity; *c.g.*, cerebral ganglion; *ph.gl.*, pharyngeal gland-cell.

Fig. 19.—Longitudinal section of the anterior portion of an embryo about 6 mm. in length, showing the primary pharyngeal nephridia and the buds of the secondary ones arising on the ducts. *d.v.*, dorsal vessel; *lat.oe.v.*, lateral oesophageal vessel.

Fig. 20.—Three embryos of typical ages of Pheretima rodricensis, shown in natural size. *A* is an embryo about 4 mm. in length, from sections of which figs. 5, 8, 9, and 22 are taken. *B* is an embryo about 6 mm. in length, from sections of which figs. 17, 18, and 23 are taken. Whole mounts shown in figs. 11, 12, 13, 14, and parts of 15 are taken from embryos of about this age. *C* is an embryo about to hatch out of the cocoon about 17 mm. in length. Figs. 16, 19, 20, and 21 are taken from whole mounts or sections of embryos of this age. Nat. size.